



Pilgrims at the time of the lunar festival, Perfume Pagoda outside Hanoi, Vietnam.
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*New developments in
molecular-based treatments
might improve the prognosis
for patients with brain tumors.*

Small Molecule and Monoclonal Antibody Therapies in Neurooncology

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Background: The prognosis for most patients with primary brain tumors remains poor. Recent advances in molecular and cell biology have led to a greater understanding of molecular alterations in brain tumors. These advances are being translated into new therapies that will hopefully improve the prognosis for patients with brain tumors.

Methods: We reviewed the literature on small molecule targeted agents and monoclonal antibodies used in brain tumor research and brain tumor clinical trials for the past 20 years.

Results: Brain tumors commonly express molecular abnormalities. These alterations can lead to the activation of cell pathways involved in cell proliferation. This knowledge has led to interest in novel anti-brain-tumor therapies targeting key components of these pathways. Many drugs and monoclonal antibodies have been developed that modulate these pathways and are in various stages of testing.

Conclusions: The use of targeted therapies against brain tumors promises to improve the prognosis for patients with brain tumors. However, as the molecular pathogenesis of brain tumors has not been linked to a single genetic defect or target, molecular agents may need to be used in combinations or in tandem with cytotoxic agents. Further study of these agents in well-designed cooperative clinical trials is needed.

Introduction

Malignant gliomas are challenging to treat and are associated with a high degree of morbidity and mortality. Stan-

dard treatment usually consists of cytoreductive surgery followed by radiation therapy. Based on several meta-analyses, adjuvant chemotherapy appears to add some survival benefit, but its efficacy is limited.¹⁻⁴ Standard

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Submitted December 14, 2004; accepted February 8, 2005.

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No significant relationship exists between the authors and the companies/organizations whose products or services may be referenced in this article.

Appreciation is expressed to Sharon Reynolds, Department of Neurological Surgery, University of California, San Francisco, for editorial support.

Abbreviations used in this paper: GBM = glioblastoma multiforme, EGFR = epidermal growth factor receptor, PTEN = phosphatase and tensin homolog, PI3K = phosphatidylinositol 3-kinase, PDGF = platelet-derived growth factor, VEGF = vascular endothelial growth factor, MAPK = mitogen-activated protein-kinase cascade, mAb = monoclonal antibody, NABTC = North American Brain Tumor Consortium, TMZ = temozolomide, EIAEDs = enzyme-inducing antiepileptic drugs.

chemotherapy agents used to treat malignant glioma are non-cell-cycle specific and aimed at inducing cell death. Recent advances in molecular and cell biology have expanded our understanding of the genetic and cellular alterations that may lead to brain cancer.⁵ For example, the transformation into cancer may involve amplification of oncogenes and/or loss of tumor suppressor genes. Such advances in the knowledge of these genetic and cellular alterations have been translated into new treatment agents that “target” these alterations in cell-signaling pathways and bring hope of improved prognosis for patients with brain tumors. However, this hope comes with the caution that new agents need to be studied in well-designed and properly conducted clinical trials before efficacy can be determined. This article reviews some of the molecular abnormalities found in malignant glial tumors and the related emerging molecularly targeted therapies.

Methods

We reviewed the literature on small molecule targeted agents and monoclonal antibodies relevant to biologic pathways in gliomas that have been used in brain tumor research and brain tumor clinical trials in the past 20 years. The search included English-language electronic databases, textbooks, specialty journals, proceedings, and Web sites of the North American Brain Tumor Consortium and other cooperative groups in Europe and the United States. We focused on therapies that have had the largest discussion in the literature or continue to be used in current phase I and phase II studies. Additional reviews of targeted agents for the treatment of brain tumors have been published.^{6,9}

Results

Molecular Biology

High-grade gliomas are the most common primary central nervous system neoplasms in adults. They also continue to be among the top 10 causes of cancer-related deaths despite a relatively low incidence compared to other cancers.¹⁰ Recent studies have demonstrated that glioblastoma multiforme (GBM; grade IV astrocytoma) commonly expresses molecular or genetic abnormalities that influence the signal pathways that regulate cell proliferation. For instance, a glioma cell may overexpress oncogenes such as epidermal growth factor receptor (EGFR) or contain mutations of tumor suppressor genes such as phosphatase and tensin homolog (PTEN).¹¹ Overexpression of EGFR is the most common oncogenic alteration in GBM. These gains or losses may promote cancerous behavior but also may be targets for new treatments.

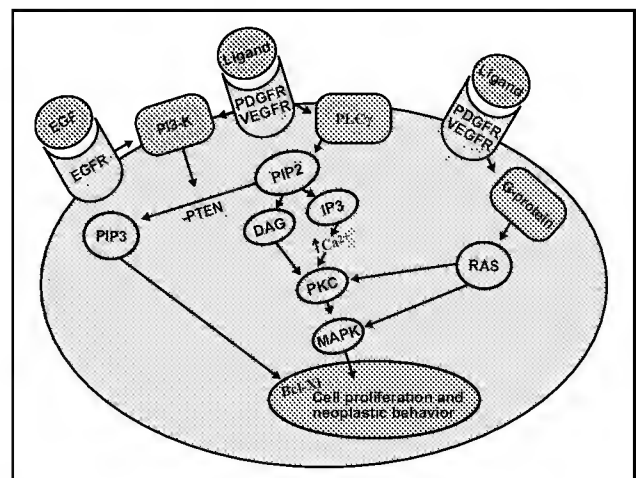
Primary GBMs develop *de novo* and usually occur in older patients who do not have a prior history of lower-grade astrocytoma. Primary GBMs generally overexpress

EGFR, a tyrosine kinase receptor with downstream effects resulting in cell proliferation and invasion. Secondary GBMs are thought to arise from lower-grade astrocytomas and typically occur in younger age groups.^{12,13} These GBMs generally do not overexpress EGFR; instead, they commonly have mutations in tumor suppressor gene p53.^{11,14}

The role of EGFR is to mediate cell growth and proliferation via activation of phosphatidylinositol 3-kinase (PI3K). PI3K facilitates the phosphorylation of phosphatidylinositol-3,4-bisphosphate (PIP2) to form phosphatidylinositol-triphosphate (PIP3). PIP3 upregulates antiapoptotic factors and promotes cell survival. PIP3 also induces kinases to phosphorylate residues that activate Akt, a protein that has antiapoptotic effects and is involved in cell proliferation.¹⁵ PTEN negatively regulates PIP3 by dephosphorylating it back to PIP2. Therefore, the PTEN gene product also indirectly regulates activation of Akt. PTEN can be mutated in GBM and further predispose the cells toward cancerous proliferation.^{16,17} In fact, mutations of PTEN are found in 45% of GBMs.¹⁶

The most common type of EGFR mutation is known as EGFRvIII. This mutant receptor is constitutively active and exists in a low-level state of autophosphorylation that induces receptor signaling. It lacks an extracellular receptor domain and cannot bind ligand. It is thus immune to the down-regulation that would occur when ligand activates a normal receptor.¹⁸

Tumor suppressor gene p53 is responsible for cell-cycle control, DNA repair after radiation damage, and induction of apoptosis, and it is mutated in approximately 50% of cancers and in 30% of gliomas.¹⁹ Mutation of tumor suppressor gene p53 results in decreased apoptosis in response to DNA damage and predisposes the cell toward neoplastic transformation.



Simplified cell pathways. From left to right are the PI3-K, PLC, and RAS pathways. EGF = epidermal growth factor, EGFR = epidermal growth factor receptor, VEGFR = vascular endothelial growth factor receptor, PDGFR = platelet-derived growth factor receptor, PI3-K = phosphatidylinositol 3-kinase, PLC = phospholipase C, PIP3 = phosphatidylinositol-triphosphate, PTEN = phosphatase and tensin homolog, PIP2 = phosphatidylinositol-3,4-bisphosphate, DAG = diacylglycerol, IP3 = inositol 1,4,5-triphosphate, PKC = protein kinase C, MAPK = mitogen-activated protein kinase.

Other glioma cell signaling pathways may also be altered and result in tumorigenesis.^{20,22} As shown in the Figure, activation of a tyrosine kinase receptor such as platelet-derived growth factor (PDGF) or vascular endothelial growth factor (VEGF) by a growth-factor ligand can result in cellular proliferation via one of two pathways. First, binding of the receptor may activate the Ras pathway (a family of membrane-associated small GTPases), which causes the cell to proliferate via the mitogen-activated protein-kinase cascade (MAPK). To function normally, Ras requires the attachment of a farnesyl or geranylgeranyl group, called prenylation. Prenylation is catalyzed by farnesyltransferase. Secondly, VEGF or PDGF may activate phospholipase C, which catalyzes the formation of diacylglycerol and inositol 1,4,5-triphosphate (IP3) from PIP2. IP3 and diacylglycerol activate protein kinase C, which may trigger the MAPK pathway directly or through the Ras pathway.

EGFR, VEGF, and PDGF may also activate the PI3K pathway, which results in the formation of PIP3. This in turn upregulates the antiapoptotic protein Bcl-x1, which helps the cell to survive. VEGF not only causes cell proliferation but also is involved in the pathways that determine endothelial proliferation and neovascularization.¹⁶ PDGF also promotes angiogenesis by inducing VEGF expression.

Ras mutations may result in constitutive action by impairing Ras sensitivity to inactivating molecules, which leads to increased cell proliferation. However, Ras mutations are rare in astrocytomas, and it is thought that upstream components of the Ras pathway contain mutations, eg, PDGF receptor.²³

New treatment strategies for patients with gliomas focus on targeting some of the above pathways. It must be

remembered that knowledge of the entire pathways and their inter-relationships is not complete and that mutations other than those discussed above may play a role in tumor development and provide possible future targets for treatment. The Table below presents a summary of the agents discussed below.

Antibodies and Ligand Conjugates

The success of monoclonal antibody (mAb) therapy depends on several factors, including the creation of non-immunogenic mAbs (ie, mAbs that do not generate a human anti-mAb antibody), the tissue dynamics of the tumor, the integrity of the targeted antigen, and the route of administration. For instance, the large molecular weight of antibodies is likely to result in inefficient drug delivery into the brain because the blood-brain barrier prevents their passage into brain parenchyma. For this reason, mAb therapy is often delivered intratumorally rather than systemically. Such intratumoral delivery is generally done via catheter or convection-enhanced delivery methods and might avoid systemic targeting and toxicity. It can be performed into the tumor itself or after resection.

Improved technology has been used to identify several glioma antigens that have been used to develop specific mAbs. For example, mAbs against EGFRvIII have been studied in vitro and in vivo.^{24,26} Preclinical studies have revealed that these antibodies likely promote receptor internalization, resulting in decreased downstream signaling. Some antibodies can also inhibit DNA synthesis and induce complement-mediated and antibody-dependent cell-mediated cytotoxicity.²⁷ A promising agent is cetuximab, a chimeric human-mouse antibody. Cetuximab binds to EGFR and prevents ligand binding, thereby blocking ligand-induced tyrosine kinase activation and stimulating receptor internalization. Additionally, cetuximab appears to induce apoptosis and inhibit angiogenesis in vitro.^{28,29} As of this review, cetuximab has not been used in clinical trials involving patients with brain tumors since delivery strategies are still being studied.

Several clinical trials of other mAbs in patients with brain tumors have offered mixed results. mAb R3 recognizes EGFR and inhibits tyrosine kinase activation. A phase I clinical trial in 9 patients with malignant gliomas or meningiomas failed to reveal any major objective antitumor responses; however, 8 patients had stable disease at their 6-month evaluation, and 2 patients remain alive 4 years after mAb therapy.³⁰

In a prospective phase I/II trial, EMD 55900, a murine mAb directed against the EGF receptor, was administered intravenously at tumor recurrence to 16 patients previously treated with surgery, radiation therapy, and chemotherapy for high-grade glioma. No measurable tumor regression was reported, and all patients had progressive disease by 3 months.³¹

Phase I and II clinical trials of the ¹³¹I-labeled murine antitenascin monoclonal antibody 81C6 have also been

**Selected Small Molecule Targeted Agents
Being Studied in Patients With Glioma**

Class	Agent
Monoclonal antibodies	Cetuximab
	R3
	EMD 55900
Toxin-linked conjugates	TGF-PE38
	IL-13-PE38
EGFR tyrosine kinase inhibitors	Gefitinib (Iressa)
	Erlotinib (Tarceva)
	Imatinib mesylate (Gleevec)
PI3K inhibitors	Wortmannin
	LY294002
Mammalian target of rapamycin inhibitors (mTORs)	Rapamycin
	CCI-779
	RAD001
Farnesyltransferase inhibitors	R111577
	SCH66336
Antiangiogenic agents	Thalidomide
	CC-5103
	PTK787
Anti-invasive agents	Cilengitide
	Marimastat
Cell growth and migration inhibitor	Accutane

conducted in patients with newly diagnosed malignant glioma.^{32,34} Tenascin is an extracellular matrix glycoprotein that is expressed in malignant gliomas but not in normal brain tissue. Reardon et al³³ reported on 33 patients with previously untreated malignant gliomas who were treated in the phase II trial with ¹³¹I-labeled murine 81C6 injected directly into a surgically created resection cavity. Patients then received conventional external-beam radiation therapy followed by a year of alkylator-based chemotherapy. Median survival for all patients and for those with GBM was 86.7 and 79.4 weeks, respectively. After accounting for established prognostic factors such as age and Karnofsky performance status, the authors concluded that the median survival achieved with ¹³¹I-labeled 81C6 exceeded that of historical controls treated with conventional radiation therapy and chemotherapy, and they suggested that a randomized phase III study is warranted.

In an effort to increase effectiveness, mAbs may be conjugated with drugs, toxins, or radioisotopes. For example, Grana et al³⁵ described a three-step avidin-biotin pre-targeting approach to target yttrium-90 biotin to tumor cells in patients with high-grade glioma. In the first step, the patient is injected with biotinylated mAbs against tenascin (a known glioma antigen). Second, streptavidin is injected, which binds to the biotinylated antibodies on the tumor. Third, radiolabeled yttrium-90 biotin is injected and binds the streptavidin. The authors of this study claim that this approach minimizes adverse effects, delays tumor progression, and prolongs time to relapse and overall survival.

In patients with GBM, mAbs against EGFR and EGFRvIII have been used to deliver ¹²⁵I and have been proposed for use in phase III studies.^{36,37} This approach has the possible benefit of radiation penetrating into residual tumor cells. Conversely, it is associated with the risk of increased toxicity to normal tissue.

Toxin-linked conjugates combine tumor-targeting regions of antibodies and a bacterial or plant toxin. Ingestion of the antibody by the receptor allows localized delivery of the toxin. Transforming growth factor alpha PE38 (TGF α -PE38), composed of TGF α and a mutated form of the *Pseudomonas* exotoxin termed PE38, has been shown to be toxic to cells in proportion to EGFR expression.³⁸ A recently completed phase I trial of TGF α -PE38 delivered by convection-enhanced delivery to patients with recurrent malignant brain tumors has demonstrated no systemic toxicities, with an overall median survival of 23 weeks.³⁹

Compared with normal brain tissue cells, human malignant glioma cells express higher levels of interleukin-13 receptor (IL-13R). However, whether this receptor is expressed in situ has not been carefully examined.⁴⁰ A recombinant fusion protein (IL-13-PE38QQR), composed of IL-13 and a mutated form of *Pseudomonas* exotoxin, has been developed and is currently being studied in brain tumor clinical trials. Binding of IL-13-PE38QQR to the IL-13R permits internalization of the recombinant toxin, resulting in selective and potent cytotoxicity. The IL-13-PE

cytotoxin was found to be toxic to human GBM cells in vitro while normal cells were relatively spared.⁴¹ Based on these encouraging preclinical studies, several phase I clinical trials in adults with recurrent malignant glioma have been initiated that involve convection-enhanced delivery of the cytotoxin.⁴² Thus far, results have not been published. Although survival was not the primary end point of these phase I studies, prolonged survival times have been observed. In fact, IL-13-PE38QQR is now being studied in a multisite phase III trial, randomizing patients with recurrent GBM to convection-enhanced delivery of IL-13-PE38QQR or implantation of proliferosan 20 with carmustine wafers (Gliadel).⁴³

Tyrosine Kinase Inhibitors

Several synthetic EGFR inhibitors have been tested in brain-tumor clinical trials, including ZD1839 (gefitinib; Iressa) and OSI-774 (erlotinib; Tarceva). These agents compete with ATP for binding to EGFR within the tyrosine kinase domain of the receptor and thereby inhibit downstream signaling through EGFR.

Gefitinib inhibits tumor growth and reduces angiogenic growth factor production. It has been used in many preclinical studies in various cancers.⁴⁴ Clinically, the North American Brain Tumor Consortium is currently testing gefitinib alone (NABTC 00-01) or combined with temozolomide (TMZ) (NABTC 01-02) for patients with recurrent GBM.^{45,46} Full results are pending. The Radiation Therapy Oncology Group (RTOG) has begun a trial of gefitinib and radiation therapy in patients with newly diagnosed GBM. The National Cancer Institute (NCI) and Duke University have published results from a phase II trial using gefitinib as a single agent at first relapse for patients with GBM.⁴⁷ Patients from this study had a median overall survival time from treatment initiation of 39.4 weeks. Adverse events were generally mild and consisted mainly of skin reactions and diarrhea. The authors concluded that further study of this agent at higher doses is warranted.

Erlotinib has also been tested in several clinical trials. Prados et al⁴⁸ showed acceptable toxicity and encouraging preliminary results in a phase I trial combining erlotinib with TMZ in patients with malignant glioma. It should be noted that the pharmacokinetics of erlotinib were altered by enzyme-inducing antiepileptic drugs (EIAEDs). A phase I/II trial using erlotinib in patients with recurrent malignant gliomas is ongoing (NABTC 01-03).⁴⁹ Another ongoing phase II trial is treating patients with newly diagnosed GBM with erlotinib, TMZ, and concurrent radiation therapy followed by adjuvant TMZ and erlotinib.⁵⁰ A phase II study involving 30 patients with recurrent GBM treated with erlotinib alone found that 6 patients had a greater than 50% reduction in tumor size and 7 patients had stable disease for 3 months or more. More than 10% of patients were progression free at 1 year after treatment.⁵¹ Lastly, a phase II study of

erlotinib plus TMZ administered during and following radiation therapy in patients with newly diagnosed GBM or gliosarcoma has started enrollment at the University of California, San Francisco.

PDGF tyrosine kinase inhibitors also have been tested in clinical trials. STI-571 (imatinib mesylate; Gleevec) has shown activity against glioblastoma cell lines in pre-clinical in vitro studies.⁵² The NABTC has tested STI-571 in patients with recurrent malignant glioma in a phase I/II clinical trial (NABTC 99-08)⁵³ and has an ongoing study of this agent in patients with meningioma. The European Organization for the Research and Treatment of Cancer is also testing STI-571 in a trial, EORTC-16011, which treats patients with low-grade or high-grade glioma who have relapsed after prior radiation therapy or chemotherapy.⁵⁴ The objectives of these studies are similar and include assessing the therapeutic activity of STI-571 (in terms of objective response and progression-free survival at 6 months) and the safety and pharmacokinetics of this drug in these patients.

Inhibition of the PI3K/Akt Pathway

Research has confirmed that cell growth and survival are influenced by PI3K and Akt.^{55,56} In gliomas, these pathways are likely overactive due to upstream activation of growth-factor receptors such as EGFR. PTEN is an important regulator of the EGFR pathway and antagonizes PI3K effects.⁵⁷ PTEN mutations are present in 15% to 40% of GBM, and they contribute to the high activity of the PI3K/Akt pathway in these tumors.⁵⁸ Several agents that inhibit the PI3K pathway are being studied. The PI3K inhibitor LY294002 has shown activity against cultured glioma cells but has not yet moved into clinical studies. In an in vitro study, cells treated with LY294002 were noted to have reduced concentrations of Akt.⁵⁹ Wortmannin is another PI3K inhibitor and appears to be a radiation sensitizer of glioma cells but has also not yet moved into clinical testing.⁶⁰

To date, direct inhibitors of Akt have been difficult to develop and have not been tested in glioma clinical trials. An alternative approach to developing therapies that directly inhibit Akt has been to develop agents that are directed at the mammalian target of rapamycin (mTOR) pathway, which is activated by Akt and is involved in regulation of protein synthesis and cell growth.⁶¹ The classic mTOR inhibitor is rapamycin, which was initially developed as an immunosuppressant for patients undergoing transplantation. This agent works by forming a complex that binds to mTOR and inhibits its kinase activity, resulting in G₁ cell-cycle arrest.⁶² Preclinical studies have shown that rapamycin is cytostatic against xenografts of glioblastoma and medulloblastoma.⁶³ Compared with PTEN-positive tumors, PTEN-negative tumors appear to be more sensitive to inhibition by rapamycin.⁶² Rapamycin is relatively unstable in solution and therefore has not been used in many clinical trials. Instead, more soluble ester

analogs of rapamycin (eg, CCI-779) have been studied. CCI-779 has been shown to inhibit GBM proliferation in vitro and is currently being tested in several clinical trials.⁶⁴ Chang et al⁶⁵ recently published results of a phase I clinical trial using CCI-779 in patients with recurrent malignant glioma who were also taking EIAEDs and found it to be well tolerated. A phase I/II trial has begun through the NABTC in patients with malignant gliomas, involving stratification between patients taking EIAEDs and those not taking EIAEDs. Full results are pending completion of the trial. Other mTOR inhibitors, such as rapamycin analog drug (RAD001), are also now being investigated in pre-clinical research and in clinical trials

Inhibition of the RAS/MAPK Pathway

Ras is an intermediate G protein in the cell-signaling pathways involved in cell growth, membrane activity, and apoptosis.⁶⁶ Before Ras becomes active, it must undergo several posttranslational modifications, including farnesylation, catalyzed by farnesyltransferase. Alterations in Ras lead to its constitutive activity. Ras mutations in gliomas are relatively rare in comparison with other cancers.⁶⁷ However, despite the lack of mutation, there still exists an increased expression of Ras in GBM cell lines in comparison with normal brain cells.⁶⁸ The current theory is that activation of Ras in malignant gliomas may be due to the overactivity of membrane tyrosine kinase receptors.⁶ Some farnesyltransferase inhibitors (FTIs) have been proven to inhibit the growth of multiple tumors, presumably by blocking Ras-mediated cell signals.⁶⁹ Synthetic FTIs such as R115777 (tipifarnib) and SCH66336 (lonafarnib) are being tested in clinical trials. Both of these demonstrated positive results in preclinical studies.⁷⁰ The NABTC has begun two trials involving R115777. The first is a phase I trial of R115777 with radiation therapy in patients with newly diagnosed GBM.⁷¹ The second, which has closed accrual, is a phase I/II trial of R115777 alone in patients with recurrent malignant glioma.⁷² SCH66336 is currently being used in a multi-institutional phase I study to treat children who have recurrent or progressive brain tumors.⁷³

Angiogenesis and Invasion

Angiogenesis is vital to tumor growth and is a key feature in pathologic diagnosis of GBM. The process of angiogenesis is a delicate balance between positive angiogenic factors (eg, VEGF, PDGF) and inhibitory factors (eg, thrombospondin, angiostatin). Overexpression of PDGF or other growth factors leads to the upregulation of VEGF and the remodeling of the extracellular matrix to permit endothelial migration.^{74,75} Angiogenesis is also regulated by integrin-mediated signaling. Integrins are cell-surface adhesion molecules often overexpressed in gliomas that mediate cell adhesion, migration, and invasion into the surrounding tissue.^{76,77} Invasion into the extracellular matrix (ECM) is further mediated by matrix metallopro-

teinasases (MMPs), which are tumor-secreted proteases that degrade ECM proteins, thereby providing room for tumor infiltration. These pathways are targets for antiangiogenic or anti-invasive therapy.^{78,79}

Though its mechanism of action is not completely understood, thalidomide is a promising antiangiogenic factor. It is thought that thalidomide inhibits or interferes with integrin receptors and inhibits neovascularization caused by VEGF. In a phase II trial, Fine et al⁸⁰ treated patients with recurrent high-grade glioma with thalidomide; it was well tolerated and median survival was 28 weeks. A group from Australia also recently completed a phase II trial of thalidomide for the treatment of patients with recurrent GBM.⁸¹ The median survival from this study was 31 weeks, and the 1-year survival rate was 35%. This study also found that thalidomide is a well-tolerated drug that may have some activity in the treatment of recurrent glioblastoma. In addition, the authors suggested that the dose of thalidomide required to achieve optimal biological impact might be better defined once reliable surrogate end points have been established. Thalidomide has also been studied in combination with more traditional chemotherapeutic agents. For instance, the neuro-oncology group at the University of California, San Francisco recently completed a phase II study of TMZ and thalidomide with radiation therapy for the treatment of patients with newly diagnosed GBM.⁸² This combination was relatively well tolerated, with favorable survival outcome for patients with GBM when compared to patients not treated with adjuvant chemotherapy and similar to those who received adjuvant nitrosourea chemotherapy. However, it was unclear what added advantage thalidomide has in combination with TMZ for this patient population.

Other antiangiogenic factors are in development, including CC-5103, an analog of thalidomide. A phase I trial of CC-5103 in adults with recurrent high-grade glioma or other refractory central nervous system malignancies is currently underway.⁸³ Treatment with CC-5103 has been well tolerated, with no sedation, constipation, peripheral neuropathy, or skin rash, all of which are common side effects of thalidomide treatment.

Another potential antiangiogenic agent is PTK787, an inhibitor of VEGF receptor tyrosine kinases that interferes with VEGF-dependent glioma vascularization and growth. Preclinical studies have demonstrated that PTK787 appreciably halts VEGF-mediated glioma growth by inhibition of neovascularization and proliferation.⁸⁴ Phase I trials testing an oral formulation of PTK787 administered once daily are ongoing. In one trial, PTK787 is being used alone in patients with recurrent GBM.⁸⁵ Another clinical trial combines PTK787 with TMZ or lomustine in patients with recurrent GBM.⁸⁶

As mentioned above, integrin signaling is needed for angiogenesis, cell migration, and invasion. Cilengitide, also known as EMD 121974, is an integrin antagonist that has

been studied in other solid tumor types and is currently being studied in phase I brain tumor trials.^{87,88} Preclinically, cilengitide has suppressed glioblastoma and medulloblastoma cell lines implanted in nude mice.⁸⁹ The NCI is conducting a phase I/II randomized trial using high-dose or low-dose cilengitide and radiation therapy in patients with newly diagnosed GBM.⁹⁰ Results are pending completion of the trial.

The MMPs degrade the extracellular matrix and thereby enable tumor invasion and growth.⁷⁸ MMP inhibitors have been developed and tested preclinically and in patients with brain tumors. One MMP, marimastat, reduced cell invasion during in vitro testing in cultured glioma cells.⁹¹ A recent phase II trial tested marimastat in combination with TMZ in patients with recurrent GBM.⁹² Joint and tendon pain were the major therapy-related toxicities and were reported in 47% of patients, with 5 patients removed from the study because of intolerable joint pain. The combination of TMZ and marimastat resulted in a progression-free survival at 6 months that exceeded their historical control group by 29%. The authors called for further study in patients with recurrent GBM and also for a study of therapy-induced joint pain. A phase II study of high central-dose Gamma Knife radiosurgery and marimastat in patients with recurrent malignant glioma failed to reveal a survival advantage in patients with GBM over historical controls, but it showed a small advantage in patients with anaplastic astrocytoma.⁹³ Other MMP inhibitors are in development.⁹⁴

Differentiating Agents

Another agent that shows activity against malignant gliomas is 13-*cis*-retinoic acid (13-CRA), but like thalidomide, its mechanism of action is unclear. In preclinical models, retinoids inhibit cell proliferation and migration and induce apoptosis in glioblastoma cell lines.^{95,96} Laboratory studies have revealed that 13-CRA renders glioma cells more susceptible to radiation.⁹⁷ Additionally, multi-agent combinations containing retinoids and alkylators were shown to be active in several types of cancer.^{98,99} Based on these preclinical data, several brain-tumor clinical trials have been conducted using 13-CRA as a single agent or in combination with TMZ. A single-arm phase II study of 13-CRA in patients with recurrent malignant glioma reported a median survival of 58 weeks, with patients experiencing only mild to moderate toxicity.¹⁰⁰ A phase II study showed the combination of 13-CRA and TMZ treatment to be active in patients with recurrent malignant gliomas. The authors suggested that the potential additive benefit was based on the different mechanisms of biologic activity.¹⁰¹ More recently, a phase II study in patients with newly diagnosed supratentorial GBM used the combination of TMZ and 13-CRA given daily with conventional radiation therapy, followed by adjuvant therapy with TMZ and 13-CRA.¹⁰² The combined therapy was relatively well tolerated, and survival was improved

compared to patients treated with radiation alone, without adjuvant chemotherapy. However, no survival advantage was seen compared to historical studies using radiation either with adjuvant nitrosourea chemotherapy or with TMZ alone. Fenretinide, a synthetic retinoid that induces apoptosis in several types of malignant cells, has also been tested in a phase II clinical trial in patients with recurrent malignant glioma.¹⁰³ Fenretinide did not demonstrate any efficacy in the patients tested at the dose and schedule used in this study. However, authors of the study thought that this lack of efficacy might be due to doses that were too low, and they called for further testing at higher doses.

Conclusions

The increasing knowledge of cell-growth signaling pathways and of the role of oncogenes and tumor suppressor genes in tumorigenesis is critical to developing new molecular-based approaches for the treatment of brain tumors. Hopefully, these new therapies will lead to improved prognosis for patients with brain tumors. To appropriately evaluate the efficacy of these new agents, the neuro-oncology community may need to redefine clinical trial design and strategy. To date, clinical trial design has been based on the evaluation of traditional cytotoxic chemotherapy, with determination of the maximum tolerated dose in phase I studies and clinical efficacy in phase II studies. Many of the above-mentioned targeted agents are not necessarily cytotoxic and may require different methods to evaluate appropriate dose, effectiveness, response, or stability. Unlike cytotoxic agents, which act on DNA, these novel therapies have different targets such as membrane receptors, signaling pathways, and proteins or factors important in cell cycle regulation or in angiogenesis. As such, these agents may inhibit tumor progression rather than cause tumor regression. Novel agents may also be more selective and less toxic to normal tissue. Considering these points, the phase I calculated dose of the targeted agent needed to achieve tumor inhibition may not be the dose that produces significant organ toxicity. Therefore, while the goal of phase I trials of targeted agents remains the determination of the recommended phase II dose, this dose is likely to be determined by biological end points and not necessarily by the maximum tolerated dose. Thus, new protocol designs may require tissue sampling or surrogate markers that indicate molecular changes.¹⁰⁴

Additionally, molecular agents may prevent tumor growth without shrinking the tumor. Thus, response measured as tumor regression may not be an appropriate phase II end point for these agents. Possible end points for molecularly targeted agents could include time to tumor progression, change in tumor markers, and measures of target inhibition. Also, for accurate efficacy assessment, pre-treatment molecular profiling of tumors may need to be performed to determine if the mechanism of drug is

appropriate to the genetic alteration found within the tumor. Also important to consider is that the molecular pathogenesis of brain tumors has not been linked to a single genetic defect or target, so a single molecular agent cannot be expected to be an effective treatment. Thus, these new agents may need to be studied in combinations or in tandem with cytotoxic agents. This will require a combination of clinical trial designs to properly determine patient benefit. Finally, although many advances have occurred in the understanding of the molecular biology of gliomas, much is left to discover.

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